

DESCRIPTION

Source Chinese Hamster Ovary cell line, CHO-derived human Wnt-3a protein
Ser19-Lys352
Accession # NP_149122

N-terminal Sequence Analysis Ser19

Predicted Molecular Mass 37.4 kDa

SPECIFICATIONS

SDS-PAGE 40 kDa, reducing conditions

Activity Measured by its ability to induce alkaline phosphatase production by MC3T3-E1 mouse preosteoblast cells.
The ED₅₀ for this effect is 5.00-25.0 ng/mL.

Measured by its ability to induce Topflash reporter activity in HEK293T human embryonic kidney cells.
The ED₅₀ for this effect is <500 ng/mL. Protein concentrations should be titrated based on cell type and if appropriate, passage number of the cell line.

Optimal concentrations should be determined by each laboratory for each application.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >75%, by SDS-PAGE under reducing conditions and visualized by silver stain.

Formulation Lyophilized from a 0.2 µm filtered solution in PBS, EDTA and CHAPS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution Reconstitute at 200 µg/mL in sterile PBS.

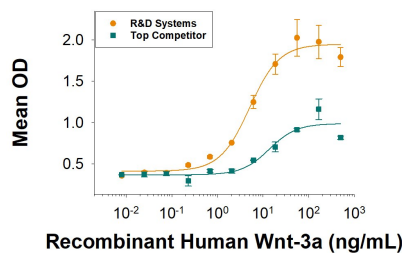
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

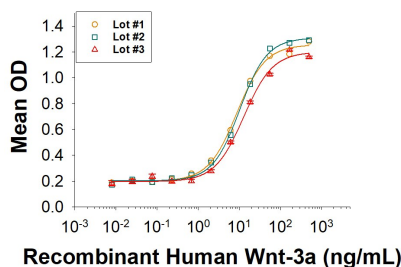
DATA

Bioactivity



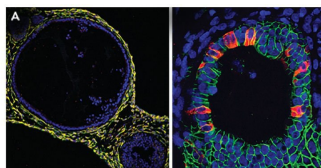
Recombinant Human Wnt-3a Protein Bioactivity Recombinant Human Wnt-3a (Catalog # 5036-WN/CF) induces alkaline phosphatase production by the MC3T3-E1 mouse preosteoblast cell line. The ED₅₀ is 1.7-fold better with more than twice the maximum response compared to the top competitor's Wnt-3a.

Bioactivity



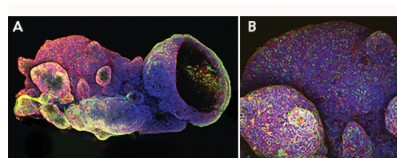
Recombinant Human Wnt-3a Protein Lot to Lot Consistency The lot-to-lot consistency of Recombinant Human Wnt-3a (Catalog # 5036-WN/CF) was assessed by testing the ability of three independent lots of the protein to induce alkaline phosphatase production in the MC3T3-E1 mouse preosteoblast cell line. Each trace on the graph represents data obtained from Recombinant Human Wnt-3a from a different manufacturing run. The ED₅₀ for this effect is 5.00-25.0 ng/mL.

Cell Culture



iPSC-derived Human Intestinal Organoids Cultured using Recombinant Human Wnt-3a Protein. iPSC-derived human intestinal organoids were cultured using Cultrex™ UltiMatrix RGF Basement Membrane Extract (Catalog # BME001-05) and intestinal organoid culture medium, which includes Recombinant Human Wnt-3a (Catalog # 5036-WN/CF), Recombinant Human EGF (Catalog # 236-EG), Recombinant Human Noggin (Catalog # 6057-NG), Recombinant Human R-Spondin 1 (Catalog # 4645-RS), along with the other reagents listed in the intestinal organoid culture medium recipe in the [human intestinal organoid culture protocol](#). (A) Human intestinal organoids were stained using a Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105; green) and a Goat Anti-Human/Mouse Desmin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3844; red) to visualize myofibroblast cells and counterstained with DAPI (Catalog # 5748; blue). (B) Human intestinal organoids were stained using a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF748; green) and a Mouse Anti-Human MUC2 Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-44431; red) and counterstained with DAPI (Catalog # 5748; blue).

Cell Culture



Adult Stem Cell-derived Human Descending Colon Organoids Cultured using Recombinant Human Wnt-3a Protein. Adult stem cells isolated from human descending colon were embedded in Cultrex UltiMatrix RGF Basement Membrane Extract (Catalog # BME001-05) and cultured for 30 days in intestinal organoid culture medium, which includes Recombinant Human Wnt-3a (Catalog # 5036-WN/CF), Recombinant Human EGF (Catalog # 236-EG), Recombinant Human Noggin (Catalog # 6057-NG), Recombinant Human R-Spondin 1 (Catalog # 4645-RS), along with the other reagents listed in the intestinal organoid culture medium recipe in the [human intestinal organoid culture protocol](#). (A) Organoids were fixed and stained with a Mouse Anti-Human MUC2 Monoclonal Antibody (Novus Biologicals; Catalog # NBP2-44431; green) to visualize intestinal goblet cells and counterstained with a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF748; red) and DAPI (Catalog # 5748; blue). The image shown was taken at 10x magnification. (B) Organoids were fixed and stained with a Mouse Anti-Human Chromogranin A Monoclonal Antibody (Catalog # MAB90981; green) to visualize enteroendocrine cells and counterstained with a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF748; red) and DAPI (Catalog # 5748; blue). The image shown was taken at 20x magnification.

BACKGROUND

Wnt-3a is one of 19 vertebrate members of the Wingless-type MMTV integration site (Wnt) family of highly conserved cysteine-rich secreted glycoproteins important for normal developmental processes (1). Wnts bind to the cell surface Frizzled family receptors in conjunction with low-density lipoprotein receptor-related protein family receptors (LRP5 or 6) resulting in the stabilization of intracellular β -catenin levels (2). As intracellular β -catenin levels rise, β -catenin binds to TCF/LEF transcription factors leading to expression of Wnt target genes (3). Endo-IWR 1 (Catalog # 3532, # PSM1324) is a cell-permeant small molecule inhibitor of Axin turnover that suppresses Wnt signal transduction by stabilizing the β -catenin destruction complex (4). Wnt-3a is a 44 kDa secreted hydrophobic glycoprotein containing a conserved pattern of 24 cysteine residues (5). Wnt-3a has two N-linked glycosylation sites (Asn 87, Asn 298), and Ser 209 is modified with palmitoleic acid (6). Glycosylation and acylation are essential for efficient Wnt secretion and biological activity, respectively (6, 7). Human Wnt-3a shares 96% amino acid (aa) identity with mouse mouse, bovine and canine Wnt-3a, and 89%, 86% and 84% aa identity with chicken, Xenopus and zebrafish Wnt-3a, respectively. It also shares 87% aa identity with Wnt3. During embryonic development, Wnt-3a is necessary for proper development of the hippocampus, anterior-posterior patterning, somite development, and tailbud formation (9-12). Wnt-3a also promotes self-renewal of hematopoietic stem cells, neural stem cells, and embryonic stem cells (13, 14).

References:

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